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The Effect of Hydrogen Peroxide on *Leishmania amazonensis* Promastigotes

Debra O. Eluobaju

ABSTRACT

Leishmaniasis is a disease caused by the parasite *Leishmania*. The disease causes lesions to the skin and face; when visceral it becomes fatal to its host. *Leishmanias* are transmitted through the female blood-sucking sand fly into its mammalian host, where it infects macrophages. Within the macrophages, *Leishmania* differentiates from a motile, rod shaped, nonvirulent promastigotes to a non-motile, spherical shaped, virulent amastigotes. Differentiation is due to the high temperature of the mammalian host body, in addition to the low pH of the macrophage. In vitro, the cells are differentiated in Graces medium (pH 5.3) at 33°C. I investigated whether hydrogen peroxide induces differentiation in *L. amazonensis*. Four samples of *L. amazonensis* were tested, a wild-type 12-1, and three triple transfectants: DT-1 GFP, DT-2 GFP, and JP-2. The samples were incubated in 199-FBS medium with hydrogen peroxide concentrations ranging from 15mM to 500mM. The cells were stained and viewed under a brightfield microscope to determine if differentiation occurred. Some hydrogen peroxide concentrations killed the cells; no differentiation was observed at the concentrations tested.

INTRODUCTION

The parasite *Leishmania* is predominately zoonotic, being passed from the female phlebotomine sand fly to a human host or from animal to animal¹. The *Leishmania* exist in its vector in its promastigotes-undifferentiated form, in the digestive tract, where it develops and multiplies. The undifferentiated form of *Leishmania*, promastigotes, is a rod shaped parasite, with a flagellum that aids in motility. When the sand fly bites the skin of its mammalian host taking a blood meal, it releases the parasites; the promastigotes are then phagocytized by the macrophages of the host².

In response to the macrophages high temperature and acidic conditions, the promastigotes thrive and undergo differentiation into amastigotes. An amastigotes is a spherical shaped parasite, that is immotile, and lacking a flagellum. After developing and multiplying within the macrophage, the parasite lyses the cell and further infects other macrophages. The cycle is repeated when a sand fly bites the host again and ingests the infected macrophages³. When in the gut of the sand fly, the parasites then differentiate back into promastigotes and multiply, with the potential to infect another mammalian host.

In the host tissue amastigotes induce asymptomatic cases. The simple cutaneous form of *Leishmania* can be identified by the development of skin lesions, most of which are self-limited, and self-healing in a couple of

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months. The disease can also spread to the face, causing facial disfigurement. *Leishmania* becomes fatal when the infection metastasizes from the skin and becomes visceral, better known as Kala-azar. Examples of potentially fatal metastasis include movement to the bone marrow, spleen, and liver.¹

Despite recent advances in understanding both the life cycle, and the process of differentiation in *Leishmania*, we do not fully understand what triggers the differentiation of the promastigotes to amastigotes in the human host. It was previously hypothesized that the combination of the high temperature of the mammalian host body, in combination with the low pH of the macrophage induces differentiation. In vitro, currently promastigotes are differentiated to amastigotes through placing cells in Graces medium with a pH of 5.3, and incubating the cells at 33°C¹. However, newer studies reveal the possible correlation between the differentiation of *Leishmania* from promastigotes, to amastigotes and the presence of hydrogen peroxide³.

The purpose of this experiment is to test whether hydrogen peroxide induces differentiation in *L. amazonensis*, from noninfectious promastigotes, to infectious amastigotes.

METHODS

CELL PREPERATION

L. amazonensis was the species used throughout the experiment; a wild-type 12-1, and three triple transfectants; DT-1 GFP, DT-2 GFP, and JP-2 were the samples observed for responses to exposure to hydrogen peroxide. The wild-type was used because previous studies reported successful differentiation in the presence of hydrogen peroxide³. The triple transfectants were used because they were the strains other researchers were using when attempting to make vaccines for Leishmaniasis. Samples were incubated in 199-FBS at 25°C³. From each flask, 2 mL of samples of 45 million cells were taken. The samples were pelleted by centrifugation, for 5 minutes, at 4°C, at 3,500 g's. The pelleted samples were resuspended in 7

mL of 199-FBS, making the cell density 14.3 million cells per mL.

HYDROGEN PEROXIDE PREPERATION

Walgreens Hydrogen Peroxide 3% First Aid Antiseptic was used as the source of hydrogen peroxide in this experiment. Hydrogen peroxide (0.882M), 11.3µL was combined with 1mL of sterile water to make a 10mM solution.

PREPERATION OF WELLS

A sterile Cellstar 6x4 well cell culture plate was used for serial dilutions. A 50µL of the sterile H₂O₂ was plated in the first well of each of the four columns; one of the four experimental groups (2mL) was then added to each well. The remaining 6 wells in each column received 1 mL of sample. Two-fold dilutions were performed; transferring 1mL from the first well to the next, the process was repeated for all 6 wells. The concentrations of the wells were 500mm, 250mm, 125mm, 62mm, 31mm, and 15mm respectively, the first five wells contained a cell density of 14.3 million cells, with the last well containing a density of 28.6 million cells. The wells were then covered, sealed, and incubated at 25°C overnight.

SAMPLE ANALYSIS

A sample of 150µL was converted to slides by cytopspin, at 100 rpm for 3 minutes. The slides were fixed in methanol for 5 minutes and allowed to air dry for 5 minutes. Slides were then stained with giemsa, which was a mixture of giemsa buffer, 2mL, and giemsa stain, 15 drops⁴. Slides were immersed in stain for 30 minutes, followed by the flushing of slides with giemsa buffer. A cover slip was placed on the sample and the slides were blown dry. Slides were then viewed under a brightfield microscope, where the cells were photographed and counted to observe for differentiation.

RESULTS

Figure 1 represents the medium with a hydrogen peroxide concentration of 500mM. Almost all the cells in this medium died when incubated overnight. The JP-2 sample had some viable cells, and two of them had differentiated. The remaining cells died, with a couple of them lysing. All of the DT-1, and DT-2 cells died, with a few of the DT-2 cells lysing (Fig 2A). However, all the wild type 12-1 cells had completely lysed after one night of incubation in the medium (Fig 2B).

Figure 3 represents the samples reactions to the 250mM hydrogen peroxide solution. The vast majority of the cells in each sample survived but they remained in the undifferentiated promastigotes form. There was a small percent of cell death and lysis for each sample.

Figure 4, and figure 5 represent the samples when incubated in 125mM and 62mM respectively. Both mediums had an almost 100 percent survival rate, with none of the cells experiencing any level of differentiation to the amastigotes form after incubation overnight (Fig 2C). Sample incubated in 31mM and 15mM had survival rates identical to those of figure 5.

DISCUSSION

The purpose of this experiment was to determine whether differentiation in *L. amazonensis* from promastigotes to amastigotes could be induced by hydrogen peroxide. No differentiation was observed in any of the *Leishmania* samples treated in 199-FBS containing hydrogen peroxide concentrations ranging from 500 to 15mM. Despite previous research observing

differentiation in the wild type *L. amazonensis* when incubated in 199-FBS with a hydrogen peroxide concentration of 150mM, it was unrepeatable in the lab³. It was observed that when samples were placed in mediums with a H₂O₂ concentration greater than 300mM, there was a drastic level of cell death after a night of incubation. In addition, immediately when cells were placed in the medium, the normally very active cells were noticeably lethargic. When cells were incubated in concentrations of H₂O₂ below 300mM cells lived, but there were no noticeable levels of differentiation in all the samples.

It was also observed that the triple transfectants, DT-1, DT-2, and JP-2 possessed a higher level tolerance towards hydrogen peroxide than the wildtype 12-1. When the triple transfectants were incubated at a 500mM concentration majority of the cells died, but the cell walls remained intact. Whereas when the 12-1 was incubated at a 500mM concentration the cells completely lysed (Fig 2D). It is worth investigating whether the insertion of foreign DNA into the *Leishmania* cells affects its resistances to hydrogen peroxide and why.

Possible reasons of the lack of differentiation in the cells may be due to the 199-FBS medium being used. In the lab there has been trouble growing the *Leishmania* cultures to the densities that were accustomed¹. Cells densities use to reside around 100 million per mL, now they only grow to about 50 million cells per mL. The unknown component in the medium that now disables the cells to grow to high densities may have also affected the cells ability to differentiate in the presence of hydrogenperoxide.

REFERENCES

1. Chang, Kwang Po (2012) *Leishmaniases*. In: eLS. John Wiley & Sons Ltd, Chichester. 1-12
2. Petersen, Christine Anne, and M. Heather West Greenlee (2011). "Neurologic Manifestations of *Leishmania Spp.* Infection." *Journal of neuroparasitology* 2. 1-5
3. Mitra, Bidyottam, and Norma W. Andrews(2013). "IRONy OF FATE: Role of Iron-Mediated ROS

in *Leishmania* Differentiation.” *Trends in parasitology* 29.10, 489–496.

4. Goto, Keiko, et al(1978). "Factors involved in differential Giemsa-staining

of sister chromatids." *Chromosoma* 66.4, 351-359.

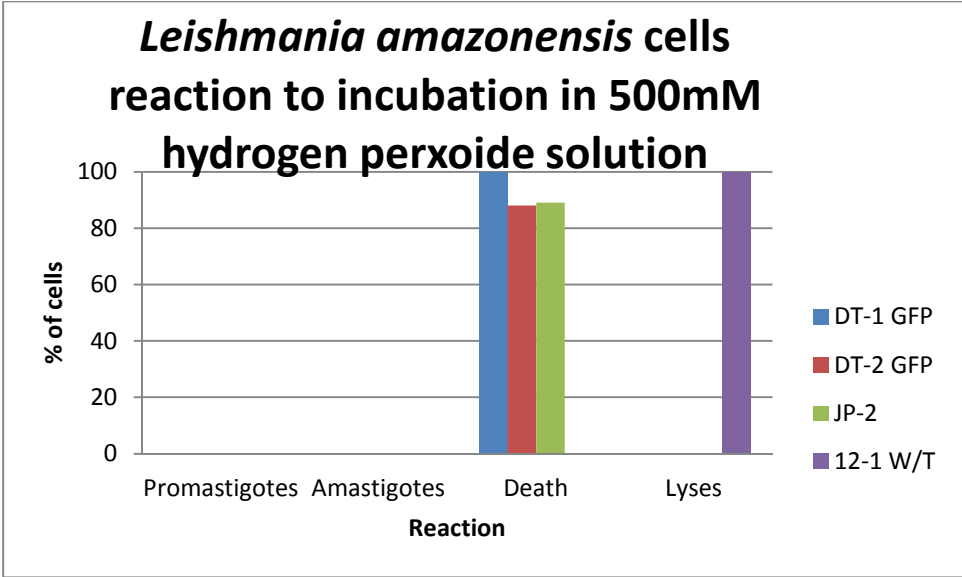
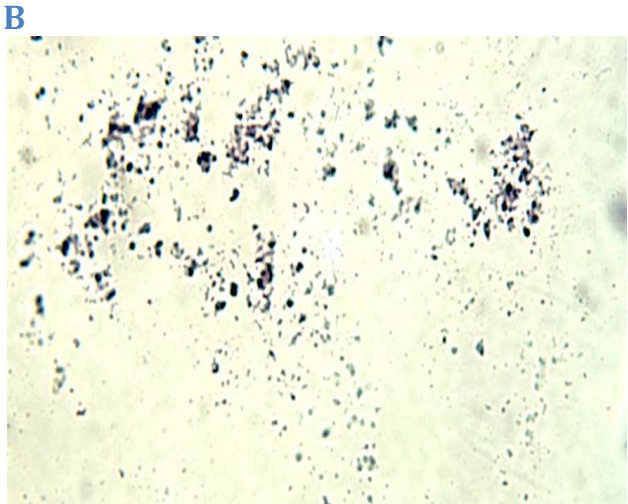
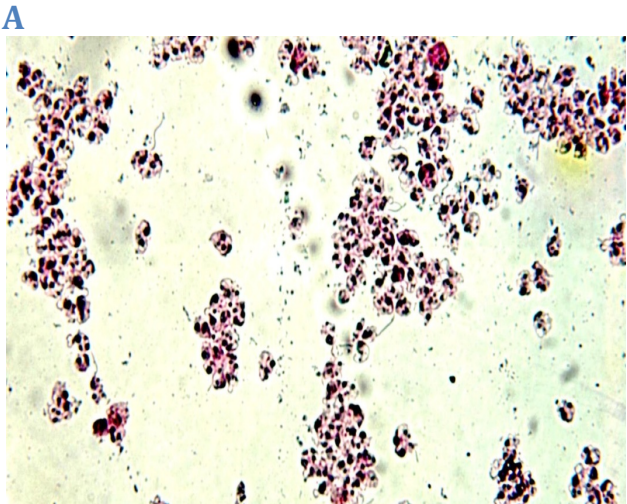
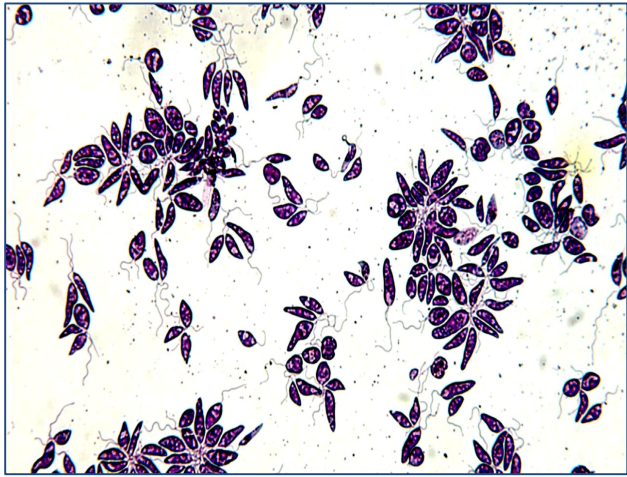


Figure 1
DT-1, DT-2, JP-2, and 12-1 samples of *L. amazonensis* after incubation in 199-FBS mediums containing 500mM hydrogen peroxide overnight at 25°C, almost complete cell death.



C



D

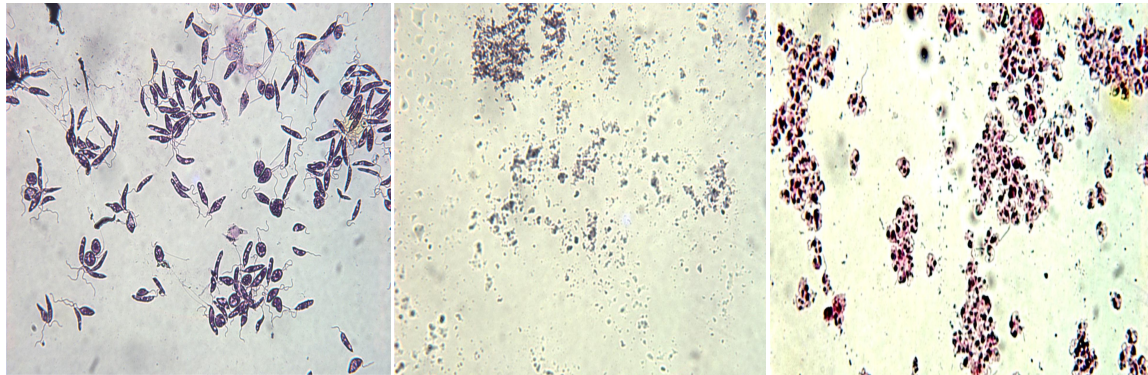
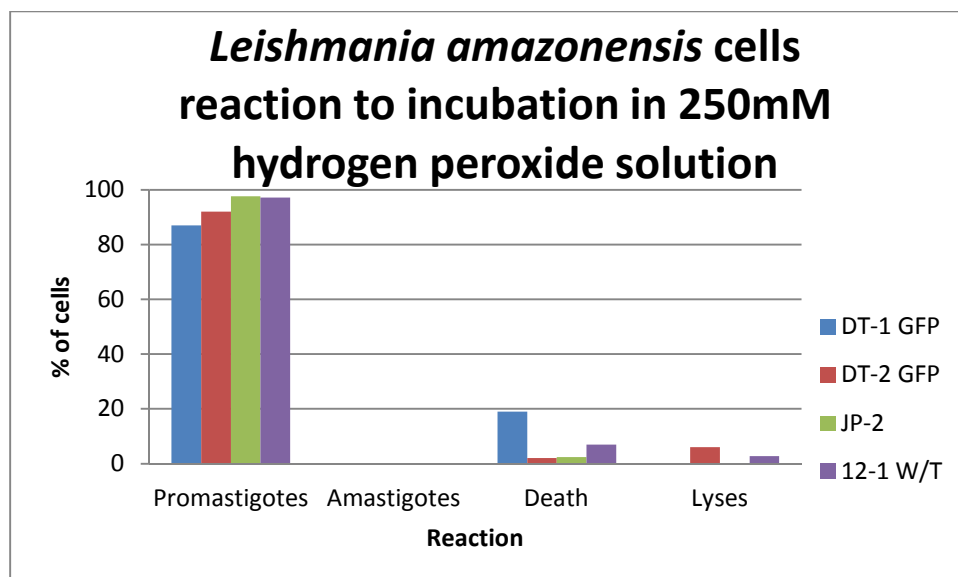


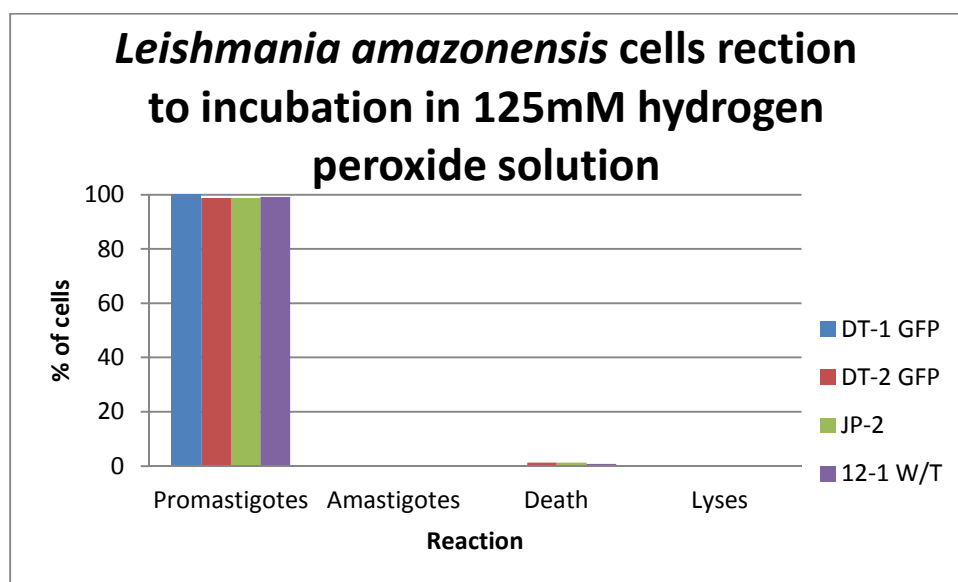
Figure 2

(A) Massive cell death in DT-2 cells after exposure to 500mM concentration. Cell walls remain intact but the cells are translucent due to the GemSia's inability to stain dead cells. (B) Complete cell death and lysis in 12-1 cells after exposure to 500mM concentration. (C) Viable and undifferentiated 12-1 promastigotes cells after exposure to 62mM. Cells have an intact cell wall, are well pigmented, and have a flagellum. (D) On the left is the control 12-1 with no exposure to hydrogen peroxide. In the center is 12-1 after exposure to 500mM concentration and there is a complete lysis. On the right is a triple transfectant (DT-2) cells after exposure to 500mM concentration. Cell walls remain intact but the cells are translucent due to the GemSia's inability to stain dead cells.

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**Figure 3**

DT-1, DT-2, JP-2, and 12-1 samples of *L. amazonensis* after incubation in 199-FBS mediums containing 250mM hydrogen peroxide overnight at 25°C, most cells are viable but undifferentiated.

**Figure 4**

DT-1, DT-2, JP-2, and 12-1 samples of *L. amazonensis* after incubation in 199-FBS mediums containing 125mM hydrogen peroxide overnight at 25°C, almost all cells are viable but undifferentiated.

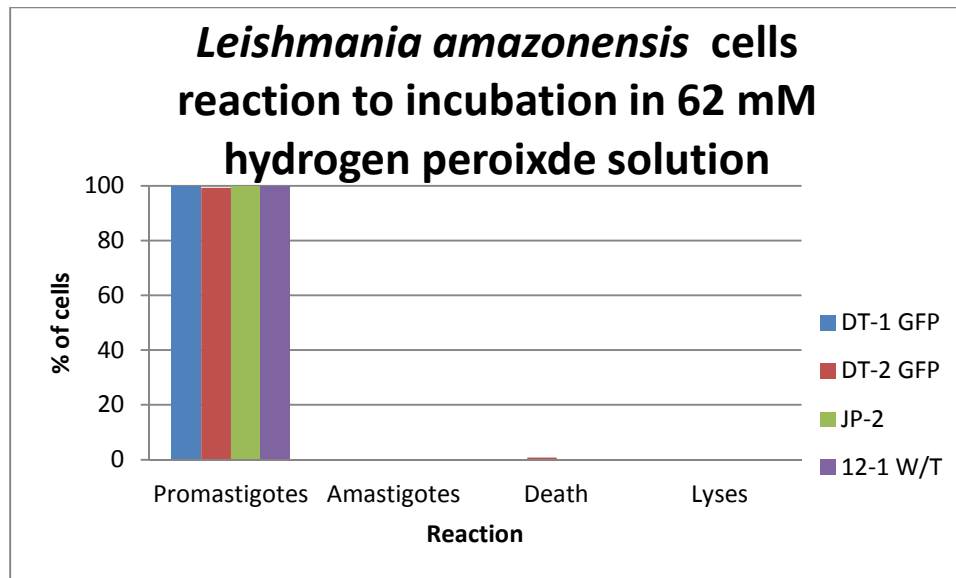


Figure 5

DT-1, DT-2, JP-2, and 12-1 samples of *L. amazonensis* after incubation in 199-FBS mediums containing 62mM hydrogen peroxide overnight at 25°C, almost all cells are viable but undifferentiated.